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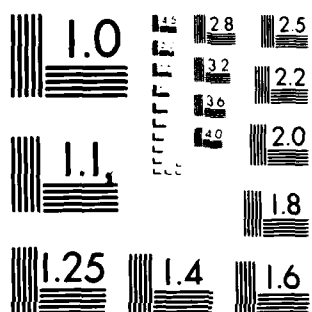
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Functional Role for Humoral Antibodies in
Leishmaniasis in Laboratory Animal

Annual and Final Report

Robert Herman

December 1982

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<p>Although stimulated and specifically and non-specifically activated macrophages are known to inhibit the replication of intracellular bacteria and some protozoan parasites <u>in vitro</u>, peritoneal macrophages harvested from C57B1/6J mice at various times following intravenous (i.v.) infection or superinfection with amastigotes of <u>Leishmania donovani</u>, supported, rather than inhibited, intracellular parasites over a nine day period. Thus, statistically significant differences, not dependent upon enhanced initial phagocytosis, were</p>		

noted in amastigotes/200 cells, % of cells infected and amastigotes/infected cell in cultures of macrophages explanted 27, 65 and 118 (but not 14) days after i.v. injection of $0.5 - 1.0 \times 10^6$ amastigotes. For example, by six days after infection of macrophages from such mice, there were 3.0-, 2.2-, and 1.6-fold more parasites/200 cells in macrophages, respectively, than in those from control mice. Similarly, macrophages obtained from mice stimulated with fluid thioglycollate medium, and those from mice infected with 200 larvae of Trichinella spiralis 39 and 49 days previously, when infected in vitro with amastigotes of L. donovani also exhibited an enhanced capacity to support the parasites.

✓ Cycloheximide ($0.5 \mu\text{g}/\text{ml}$), applied to thioglycollate-stimulated macrophage cultures for four or thirteen hours prior to infection decreased, in the latter case, the number of macrophages in culture but apparently had little effect upon parasite numbers, % of cells infected or amastigotes/infected cell.

A relationship probably exists between the method by which mice are infected (intravenously vs. intraperitoneally) and the capacity of their peritoneal macrophages to support amastigotes in vitro as noted in the results of this and other studies. In addition, a possible role for intracellular and extracellular lysosomal and non-lysosomal enzymes in the enhanced support of amastigotes from infected, superinfected, thioglycollate-stimulated and Trichinella-infected mice is considered.

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Forward:

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council

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Because cytophilic and opsonic antibody obtained from sera of C57Bl/6J mice superinfected with the intracellular protozoan parasite Leishmania donovani were shown to affect the binding and subsequent phagocytosis of the parasite by normal macrophages in vitro, it was of interest to study the binding, ingestion and replication of amastigotes (the intracellular stage) of L. donovani in cultures of peritoneal macrophages from superinfected mice whose sera contained cytophilic and opsonic antibody, as described previously (i.e., in a previous Annual Report and in Herman, 1980). Since such macrophages may have been in an activated state when harvested, the course of amastigote replication in vitro was also studied in macrophages activated by thioglycollate and in macrophages obtained from mice infected with the nematode Trichinella spiralis. The details of that study follow.

Macrophages, exposed to a wide variety of experimental conditions, may be induced to become tumoricidal and/or to have a profound effect upon the replication of intracellular bacteria and protozoan parasites. These capacities frequently reflect the means by which activation (Mackaness, 1970; Karnovsky and Lazdens, 1978) or stimulation (Ogmundsdottir and Weir, 1980) are induced. The mechanisms governing the bactericidal, parasiticidal and tumoricidal capabilities of activated and stimulated macrophages are varied (Ogmundsdottir and Weir, 1980) as are their specificities (Allison, 1978).

One characteristic of macrophage activation is its dependence upon antigenically specific stimulation of sensitized lymphocytes. Thus, inhibition of intracellular replication of Listeria monocytogenes (Simon and Sheagren, 1972), Mycobacterium tuberculosis (North, 1974), M. leprae (Godal et al., 1971) and Rickettsia tsutsugamushi (Nacy and Meltzer, 1979) have all been demonstrated after infected cells, in vitro, were exposed to specifically sensitized lymphocytes or their products. Similarly, a role for lymphokines, from specific antigen-treated sensitized splenic lymphocytes, in the induction of

intracellular parasitocidal activity has been demonstrated for Trypanosoma cruzi (Nogueira et al., 1977) and for Toxoplasma gondii (Borges and Johnson, 1975).

Macrophage activation may culminate in enhanced microbicidal or parasitocidal capacities against heterologous organisms as well. Thus, macrophages from Toxoplasma- and Besnoitia-infected mice protect against Listeria in vitro (Ruskin et al., 1969) and reciprocal in vitro cross protection has been noted in macrophages from Listeria- and Salmonella-infected mice (Blanden et al., 1966). In addition, peritoneal macrophages from mice infected with Trichinella spiralis could inhibit tumor cell DNA synthesis (Wing et al., 1977) while a decrease in tumor cell numbers could be induced by close contact in vitro with peritoneal macrophages from rats bearing the hookworm Necator braziliensis (Keller and Jones, 1971).

Results of the present study show that peritoneal macrophages, obtained from mice at various times after intravenous (i.v.) infection with amastigotes of L. donovani, and from mice which had been injected with parasites twice (superinfected mice), all demonstrated an enhanced, rather than a reduced, capacity to support these parasites in vitro. This same phenomenon was noted for peritoneal macrophages from mice infected with T. spiralis and from mice which had been injected intraperitoneally (i.p.) with fluid thioglycollate medium. Some possible mechanisms governing these observations are discussed.

MATERIALS AND METHODS

Animals

Mice used in all experiments were female C57B1/6J (Jackson Laboratories, Bar Harbor, Maine, 6 to 8 weeks old). Male golden hamsters, purchased from Charles River Lakeview, Wilmington, Mass., ranging in age from weanlings to adult, were used to maintain the 3S strain of L. donovani (Stauber, 1966) by intracardial injection of infected hamster spleen tissue (Stauber, 1958).

Infection of animals with amastigotes of L. donovani

Mice were injected i.v. with $0.5 - 1.0 \times 10^7$ amastigotes from infected hamster spleen tissue or injected with normal hamster spleen tissue as described previously (Herman, 1980). For superinfection, mice were injected i.v. with 1.0×10^7 amastigotes between 60 and 67 days after initial infection; controls were injected i.v. with normal hamster spleen tissue.

Macrophage culture

Macrophages were cultured in 8-chambered Lab-Tek culture slides (Lab-Tek Products, Div. Miles Laboratories, Inc., Westmont, IL) as described previously (Herman, 1980). Macrophages from mice which had received injection of amastigotes and macrophages from control mice inoculated with hamster spleen tissue were tested for in vitro intracellular support of amastigotes at 14, 27, 65 and 118 days after infection. At the time of macrophage harvest, spleens and livers of infected mice were weighed and Giemsa-stained impression smears of these organs prepared for determination of their parasite burdens (Stauber, 1958).

Macrophages from mice injected twice with L. donovani were explanted 11 and 24 days after the second parasite injection. Macrophages from mice injected with normal hamster spleen tissue served as controls.

Infection of macrophages in vitro

Macrophages, explanted into Lab-Tek culture slides, were exposed to a multiplicity of infection (MOI) of five amastigotes/macrophage in a volume of 0.5 ml (Herman, 1980). Cultures were incubated for 1 hour at 37°C in 5% CO₂ in air. All wells were rinsed twice with Medium 199 containing penicillin (100 units/ml) and streptomycin (100 µgm/ml); 0.5 ml of complete medium (Medium 199, 20% heat-inactivated fetal bovine serum, 100 units/ml penicillin and 100 µgm/ml streptomycin) was added to each well. Cultures were removed after 1 hour, rinsed dried, fixed with methanol and stained with Giemsa. Quantitation of parasitization of macrophages was assessed at this time and at 3, 6 and 9 days by determining the mean number of amastigotes/200 cells, the mean % of cells infected and the mean number of amastigotes/infected cell, as described previously (Herman, 1980). The medium was changed every third day.

Thioglycollate-stimulated macrophages

Thioglycollate-stimulated macrophages were obtained from the pooled peritoneal exudates of several mice, each of which had been injected i.p. 3 days previously with 2 ml of Bacto fluid thioglycollate medium (Difco Laboratories, Detroit, MI). Control macrophages were obtained from normal mice. Cell suspensions from thioglycollate-stimulated mice were adjusted to 2×10^5 cells/ml and wells of Lab-Tek culture slides were inoculated (0.5 ml/well). Cultures were incubated at 37°C overnight in 5% CO₂ in air, rinsed with Medium 199 (without serum) and infected with an MOI of 5 amastigotes of *L. donovani*, as described above.

Treatment of thioglycollate-stimulated macrophages with cycloheximide

To test the effects of cycloheximide on the in vitro support of amastigotes by thioglycollate-stimulated macrophages, 0.5 µgm of cycloheximide (Sigma Chemical Co., St. Louis, MO), in complete medium, was added to cultures of thioglycollate-stimulated macrophages for either 4 hours one day after explantation or for 13 hours starting 8 hours after explantation. In each case

untreated cultures served as controls. Following exposure to cycloheximide, treated and control cultures were rinsed twice with Medium 199 (containing penicillin and streptomycin) and then amastigotes (MOI = 5) were inoculated into all wells and the cultures incubated for 1 hour at 37°C in 5% CO₂ in air. The cultures were then rinsed, complete medium was added, and slide cultures were incubated and removed for study as described above. Medium was changed every third day.

Infection of mice with *Trichinella spiralis*

Each of 12 mice was infected, by stomach tube, with 200 larvae of *T. spiralis* prepared from infected mouse muscle (Despommier, 1974). At 39 and 49 days after infection, peritoneal macrophages from infected and normal mice were harvested, pooled separately and explanted into Lab-Tek culture slides. Exposure to amastigotes of *L. donovani* and subsequent rinsing and quantitation of parasitization of cultures was carried out as described above. At times of macrophage harvest from *T. spiralis*-infected mice, *Trichinella* infection was confirmed by the observation of larvae (at 10 X magnification) in diaphragm muscle pressed between two microscope slides.

Statistics

Statistical analysis of the data was by two-way analysis of variance.

RESULTS

In vitro infection of macrophages from L. donovani-infected mice

Peritoneal macrophages explanted from mice 27, 65 and 118 days after i.v. injection of 1×10^7 amastigotes of L. donovani demonstrated an enhanced capacity to support replication of amastigotes in vitro when compared to controls (Fig. 1). This capacity was not observed in macrophages obtained from mice 14 days after infection, but at 3 days in macrophages from mice infected for 27 days and at 6 days in macrophages from mice infected for 65 and 118 days. Similar results were also observed for % of cells infected (Fig. 2) and for amastigotes/infected cell (Fig. 3). However, in cultures of macrophages from mice infected for 118 days, a statistically significant difference in % of cells infected could not be shown, when compared to controls, until day 9. That increased phagocytic capacities of macrophages from mice infected for 27, 65 and 118 days were not responsible for these differences may be seen by the similarity, at these times, in the numbers of amastigotes/200 cells, the % of cells infected and the number of amastigotes/infected cell in macrophages from infected and control mice after exposure to parasites for one hour (Figs. 1, 2, and 3, respectively). Spleen and liver parasite burdens of mice are increasing at 14 days after infection (Table 1) and usually attain maximum burdens in these organs between 20 and 30 days after which the numbers decline (data not shown).

At all times after initial infection the total number of cells in wells of Lab-Tek culture slides, from infected and control mice, were similar. Although the numbers of cells in cultures from infected mice tended to be slightly less than those from control mice, at each time of evaluation (i.e., from 1 hour after infection of cultures through 9 days) the number of cells/well ranged from 2 to 4×10^4 , with the lower number occurring more frequently at 6 and 9 days after infection of cultures.

In vitro infection of macrophages from superinfected mice.

Macrophages, explanted from mice which had been superinfected i.v. with 1×10^7 amastigotes following an initial i.v. inoculation with this same number of parasites, were tested for their ability to support amastigotes after in vitro phagocytosis. Macrophages harvested from mice superinfected between 60 and 67 days after initial infection and their macrophages explanted 11 and 24 days later, demonstrated similar enhanced abilities to support amastigotes when compared to their respective controls. Thus, results of experiments using macrophages explanted from mice at the different times following superinfection were pooled as were those of their respective hamster spleen-injected controls (Fig. 4). The results demonstrate that significant differences between these groups in numbers of parasites/200 macrophages, % of cells infected and amastigotes/infected cell were noted only at 6 and 9 days after infection of cultures. Numbers of cells in the wells of culture slides, at all times of observation, were similar to those noted in cultures from mice receiving a single injection of parasites.

Whether mice were infected or superinfected, in no case were parasites found within their peritoneal macrophages following explantation when at least 200 cells in each of four wells of Lab-Tek culture slides were examined. Thus, when macrophages from infected and superinfected mice were used, there was no possibility of implantation of macrophages containing numbers of parasites which could influence the observed results.

In vitro infection of macrophages from thioglycollate-injected mice

Macrophages obtained from mice injected with fluid thioglycollate medium following in vitro exposure to amastigotes of L. donovani, supported the parasites to a greater degree than did macrophages from non-injected mice. By three days after exposure to parasites there were 1.4-fold more amastigotes/200 cells in thioglycollate-stimulated macrophages when compared to cells from

control mice. This difference progressed to 7.0-fold at 6 days and was 8.5-fold at 9 days (Fig. 5). Differences between the macrophage populations were noted in the % of cells infected and in amastigotes/infected cell. No substantial differences in initial phagocytosis of parasites existed between the two populations.

Since each well had been inoculated with 1×10^5 cells obtained from thioglycollate-stimulated mice (Herman, 1980), the number of cells in each culture well ranged between 2 to 5×10^4 at all times of observation and was thus similar to the numbers noted in wells containing cells from infected and superinfected mice.

In vitro infection of thioglycollate-stimulated macrophages treated with cycloheximide

Macrophages from thioglycollate-stimulated mice treated in vitro for 4 or 13 hours with cycloheximide ($0.5 \mu\text{g}/\text{ml}$) just prior to exposure to amastigotes caused no inhibition of the supportive capacity of these macrophages when compared to untreated cultures. Cultures treated for 4 hours showed no apparent effects while those treated for 13 hours demonstrated a decrease in the mean number of macrophages/well of the Lab-Tek culture slides (5.05×10^4 cells/well in untreated cultures compared to 3.41×10^4 cells/well in treated cultures), a 1.5-fold difference. In addition, macrophages in cultures treated for 13 hours exhibited decreased ability to phagocytize amastigotes in that at one hour after exposure to parasites there were 2.5-fold more amastigotes/200 cells in untreated cultures than in cells of treated ones ($p < .05$). At all times of sampling of cultures, similar differences, ranging from 1.7-fold (at 9 days) to 3.2-fold (at 2 days) were noted ($p < .05 - p < .001$). Similar differences were seen when % of cells infected and amastigotes/infected cell were compared in each case.

In vitro infection of macrophages from Trichinella-infected mice

Macrophages explanted from mice which had been infected 39 and 49 days previously with 200 larvae of I. spiralis supported amastigotes of L. donovani better than did those from non-parasitized mice (Fig. 6). This was most apparent at 6 and 9 days when there were 1.7-fold ($p < .05$) and 4.8-fold ($p < .01$) more amastigotes/200 cells, respectively, in cultures of macrophages from Trichinella-infected mice than in those from non-parasitized controls. At these times there was also a greater number of amastigotes/infected cell and, at 9 days, a greater % of cells infected (these differences being statistically significant) when the two macrophage populations were compared (Fig. 6). In agreement with the results observed when macrophages from infected, superinfected and thioglycollate-injected mice were used, there was no apparent statistically significant difference in the initial phagocytosis of parasites by macrophages from Trichinella-infected mice when compared to controls. No significant differences in the numbers of cells/well were noted between those cultures initiated with cells obtained from Trichinella-infected mice when compared to their controls. They were similar in number, at all times, to those observed in cultures of cells from infected, superinfected and thioglycollate-stimulated mice.

The results of all experiments indicate that mouse peritoneal macrophages are incapable of sustaining intracellular growth of amastigotes under the conditions of culture described. Amastigotes/200 macrophages, % of cells infected and amastigotes/infected cell decline in some cases after day 3 and in all cases after day 6.

DISCUSSION

The results of this study demonstrate that peritoneal macrophages, harvested from mice at different times following a single infection (Figs. 1, 2 and 3) or superinfection (Fig. 4) of amastigotes of L. donovani, sustained, rather than suppressed, the parasites intracellularly in vitro over a period of nine days of observation. Such enhanced support was non-specific since similar results were noted when macrophages from thioglycollate-injected mice (Fig. 5) and from mice infected with larvae of the nematode Trichinella spiralis were used (Fig. 6).

These observations differ from those of several studies in which the numbers of intracellular bacteria or protozoan parasites, in macrophages from hosts bearing homologous or heterologous infections, were reduced. In most of those cases suppression was predicated upon the action of specifically stimulated sensitized lymphocytes and/or their products (lymphokines) (Dumonde et al., 1969) while in others, such suppression followed exposure of parasite-infected macrophages to mixed lymphocyte culture (Mauel et al., 1974) or after incubation with supernatants of concanavalin A-stimulated spleen cells (Buchmuller and Mauel, 1979). In comparison, in the present work no attempt was made to activate or stimulate lymphocytes. We are aware of the possibility that peritoneal lymphocytes, harvested as part of the peritoneal cell population may already be in a state of activation or stimulation when explanted with macrophages into wells of Lab-Tek culture slides. An additional opportunity for activation of macrophages, especially in the case of cultures of cells from L. donovani-infected mice, is possible since non-adherent cell populations were not removed from the cultures until one day after explantation of cells, just prior to infection with amastigotes. We are aware also that some of the variations in the degree of parasitization of macrophages, especially in those from mice tested at 14, 27, 65 and 118 days after infection, may be reflections of

differences in parasite populations used to initiate each experiment (Hodgkinson and Herman, 1980; Hodgkinson et al., 1980).

Close inspection of the methods used in reports of enhanced microbicidal or parasiticidal capacities of macrophages from hosts infected with homologous and/or heterologous organisms may offer some explanation of the results of the present work. In most cases, the initial infection was induced by i.p. injection and macrophages were harvested at various times from this site (Ruskin et al., 1969; Blanden et al., 1966). In other studies, destruction of T. cruzi in vitro by macrophages was noted when peritoneal cells were obtained from mice injected i.p. with BCG (Kress et al., 1977) and after a secondary i.p. challenge of homologous or heterologous antigen into T. cruzi-infected mice (Nogueira et al., 1977). Of particular relevance is the report in which peritoneal macrophages, obtained from mice which had received several i.p. injections of amastigotes of L. donovani, proved unable to support intracellular growth of this parasite when compared to macrophages from uninfected mice (Miller and Twohy, 1969). That initial i.p. injections specifically activated or non-specifically stimulated lymphocytes (and subsequently macrophages) in the peritoneal cavity, resulting in inhibition of the in vitro intracellular growth of the parasites, is indeed probable. In this regard it is interesting that in vitro tumoricidal activity of macrophages from BCG-infected mice requires that the macrophages be harvested from the site of BCG-infection or reinfection (Hibbs, 1975) demonstrating that activation of macrophages is a localized event. In comparison, in the present study all infections and superinfection of mice with L. donovani were via the i.v. route, thus affording little or no opportunity for the direct exposure of peritoneal cell populations to parasites and consequently directly to lymphokines induced by parasite-lymphocyte interactions. Thus, macrophages from such i.v.-infected and -superinfected mice provide, in some manner, an intracellular milieu more conducive to parasite

replication and/or survival. Indeed, the biochemical and physiological changes in peritoneal cell populations of L. donovani- and I. spiralis-infected mice are yet to be determined.

Activated macrophages, which differ morphologically and physiologically from normal ones, may be demonstrated in animals with a variety of infections. In tuberculosis, for example, in which peritoneal exudate cells show increases in lysosomal enzymes (Dannenberg et al., 1974), activated macrophages are believed to be responsible, in part, for the killing of their intracellular bacilli (Dannenberg, 1975) and in encouraging local tissue necrosis (Dannenberg and Sugimoto, 1976). Interestingly, peritoneal macrophages obtained from L. enriettii-infected guinea pigs were unresponsive to lymphokine in the migration inhibition assay and caused a rapid increase in their level of glucose oxidation (Poulter, 1976). It is reasonable then, that the physiology of peritoneal macrophages from mice following i.v. infection by amastigotes is also altered, such change being manifested by intracellular support of parasites in vitro, first noticeable only after 14 days of infection (Figs. 1, 2 and 3). Behavior of macrophages in this manner may be congruent with acquired immunity in mice in that enhanced intracellular survival of amastigotes in vitro, first observed at 28 days following infection (Fig. 1, 2 and 3), occurs at a time when parasite burdens in spleen and liver are known to be declining (Table 1). At subsequent times of macrophage cultivation from infected mice (i.e., at 65 and 118 days postinfection), the numbers of parasites in these organs are barely detectable by impression smear. Paradoxical though the increased support of amastigotes within macrophages from infected and superinfected mice may seem, it is consonant with the previous observation that cytophilic antibody, from mice 10, 11 and 24 days following i.v. superinfection, demonstrated increased binding and subsequent phagocytosis of parasites in vitro (Herman, 1980).

A possible role may exist for the heightened enzyme levels in thioglycollate-stimulated macrophages in the observed enhanced intracellular permissiveness of macrophages for amastigote survival (Fig. 5). After i.p. injection of thioglycollate medium, mouse peritoneal macrophages show increased level of β -glucuronidase and acid phosphatase (Dy et al., 1978) and larger amounts of protein, lactic dehydrogenase, lysosomal hydrolases and especially plasminogen activator, than do normal macrophages (Schnyder and Baggiolini, 1978). Since cycloheximide inhibits significant increases in enzyme levels of macrophages following their phagocytosis of latex and carbon particles (Mørland and Mørland, 1978) and inhibits the secretion of elastase (Werb and Gordon, 1975) and collagenase (Werb and Gordon, 1975a) from thioglycollate-stimulated macrophages following phagocytosis of latex particles, the inability of thioglycollate-stimulated macrophages, treated with cycloheximide for 4 or 13 hours prior to infection with amastigotes, to suppress parasite enhancement, remains unexplained. Perhaps the intracellular presence of amastigotes so stimulated the host cells that their synthetic capabilities partially reversed the inhibitory effects of cycloheximide or a selective inhibition of enzymes by cycloheximide possibly excluded those required for parasite survival.

Changes in the extracellular milieu should be considered as possibly playing a role in the altered host cell-parasite relationship in this study. Lysosomal secretions, escaping from thioglycollate-stimulated macrophages and from those from infected, superinfected and Trichinella-infected mice when lysosomes fused with incomplete vacuoles during phagocytosis (Weissmann et al., 1971), may contain greater quantities of various enzymes (Schnyder and Baggiolini, 1977) when compared to normal mouse macrophages; the functional role for such enzymes remains unassessed in the present study. In addition, the natural heterogeneity of peritoneal cell populations (Hopper et al., 1979), known to change under the influence of thioglycollate stimulation (Dy et al.,

1978) may also do so following L. donovani or Trichinella infections so that cells, more receptive to intracellular replication of amastigotes, may prevail. Thus, cells entering and leaving the peritoneal cavity may be changed qualitatively under the influence of amastigote-parasitized cells in bone marrow.

The potential role of lysosomal enzymes in the nutrition of amastigotes, as well as the chemical composition of the ambient environment (Chang and Dwyer, 1978), is attractive when one considers that amastigotes, within macrophages in vitro, are exposed to lysosomal enzymes following phagosome-lysosome fusion (Chang and Dwyer, 1978; Alexander and Vickerman, 1975). Indeed, evidence suggests "... that intralysosomal microenvironment may be favorable for the growth of amastigotes" (Chang, 1978). Additional evidence for the permissive role of thioglycollate-stimulated macrophages comes from the recent report (Baker and Campbell, 1980) that Listeria grow considerably better in vitro in macrophages from thioglycollate-infected mice than in those from normal mice and that injection of thioglycollate medium into mice decreased their resistance to infection with Listeria.

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Table 1

Mean body, spleen and liver weights and mean parasite burdens of spleens and livers of mice at various times after i.v. infection with 1.0×10^7 amastigotes of Leishmania donovani.

Day After Infection ^(a)	Body Weight (gm)	Spleen (mg)	Liver (gm)	Total Amastigotes ^(b)	
				Spleen ($\times 10^{-4}$)	Liver ($\times 10^{-7}$)
14	20.9	108.6	1.24	8.00	7.95
27	21.2	292.1	1.79	9.24	6.14
65	23.0	213.0	1.24	4.26	.0082
118	24.6	162.4	1.28	2.91	.0189

(a) 3-5 mice/group

(b) Calculated from organ weights and impression smears (Stauber, 1958).

Legends for Figures

Fig. 1. Mean number of amastigotes/200 cells in macrophage cultures at various times after in vitro exposure to amastigotes of L. donovani. Peritoneal macrophages were obtained for culture from mice 14, 27, 65 and 118 days after i.v. infection with 1.0×10^7 amastigotes (x-----x) or normal hamster spleen tissue (o-----o) (\pm S.E.). **p < .05, *p < .01, p < .001.

Fig. 2. Mean % of cells infected at various times after exposure of macrophage cultures to amastigotes of L. donovani (\pm S.E.). All conditions are as described for Fig. 1.

Fig. 3. Mean number of amastigotes/infected cell after exposure of macrophage cultures to amastigotes of L. donovani (\pm S.E.). All conditions are as described for Fig. 1.

Fig. 4. Mean number of amastigotes/200 cells, mean % of cells infected and mean number of amastigotes/infected cell, at various times after in vitro exposure to amastigotes of L. donovani, in cultures of macrophages from superinfected (x-----x) and normal hamster spleen-injected (o-----o) mice (\pm S.E.). Mice superinfected were injected i.v. with 1.0×10^7 amastigotes between 60 and 67 days after initial infection with $0.5 - 1.0 \times 10^7$ amastigotes and their peritoneal macrophages explanted 11 and 24 days later. At all times of infection and superinfection control mice were injected with normal hamster spleen tissue. Because of the similarity obtained in macrophage cultures from superimmunized mice, these results represent data pooled from 3 experiments. **p < .05, *p < .01, p < .001.

Fig. 5. Mean number of amastigotes/200 cells, mean % of cells infected and mean number of amastigotes/infected cell, at various times after in vitro exposure to amastigotes of L. donovani, in cultures of macrophages explanted from mice injected i.p. with fluid thioglycollate medium (x-----x) and from normal (o-----o) mice (\pm S.E.). Results from pooled data of 4 experiments.

Fig. 6. Mean number of amastigotes/200 cells, mean % of cells infected and mean number of amastigotes/infected cell, at various times after in vitro exposure to amastigotes of L. donovani, in cultures of macrophages from Trichinella spiralis-infected (x-----x) and non-infected (o-----o) mice (\pm S.E.). Results obtained from cultures prepared from Trichinella-infected mice 39 and 49 days after infection were similar, compared to their respective controls, and are thus pooled. **p < .05, *p < .01, p < .001.

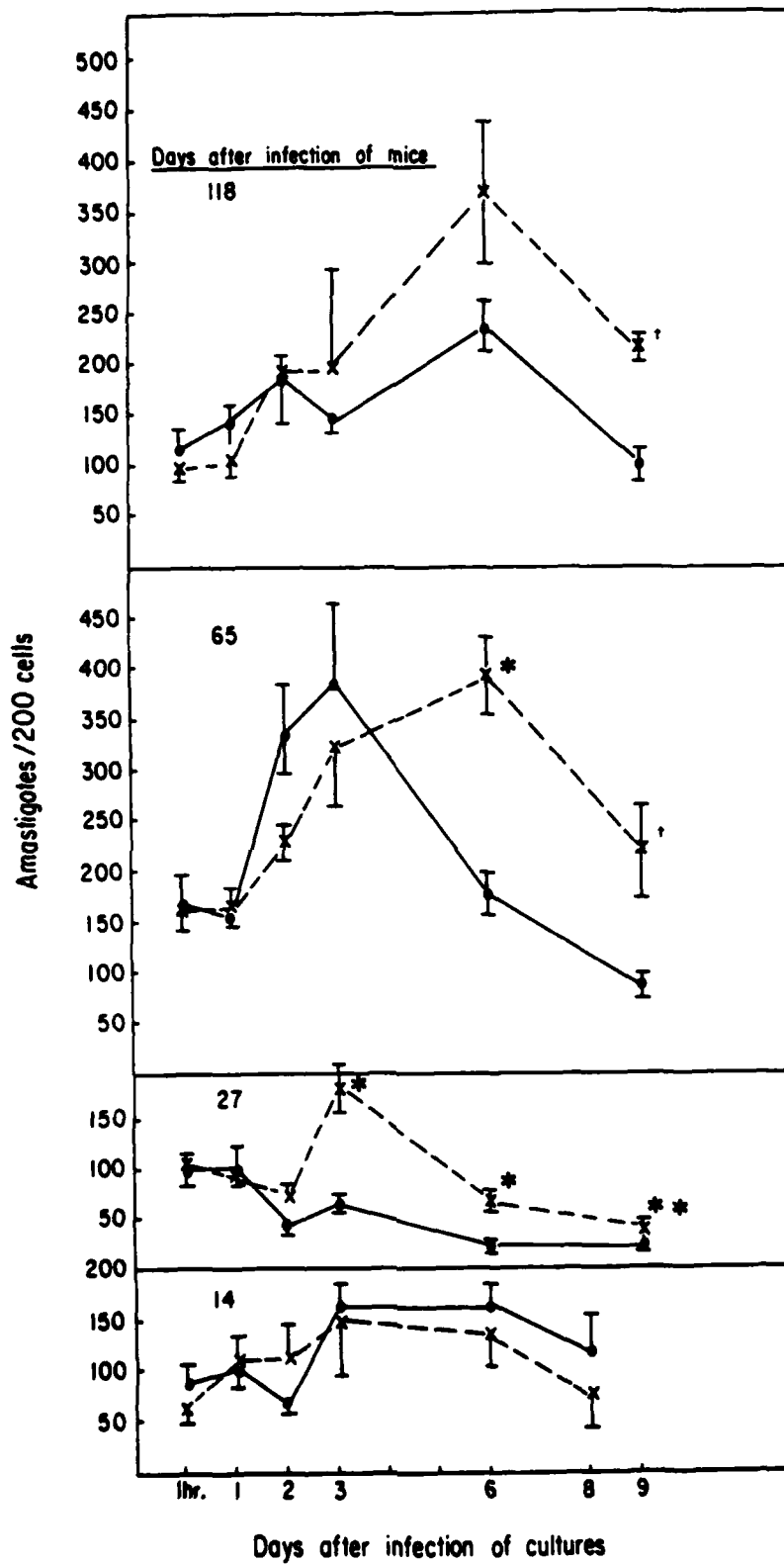
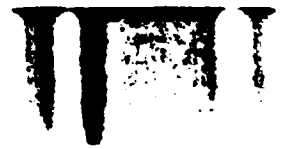
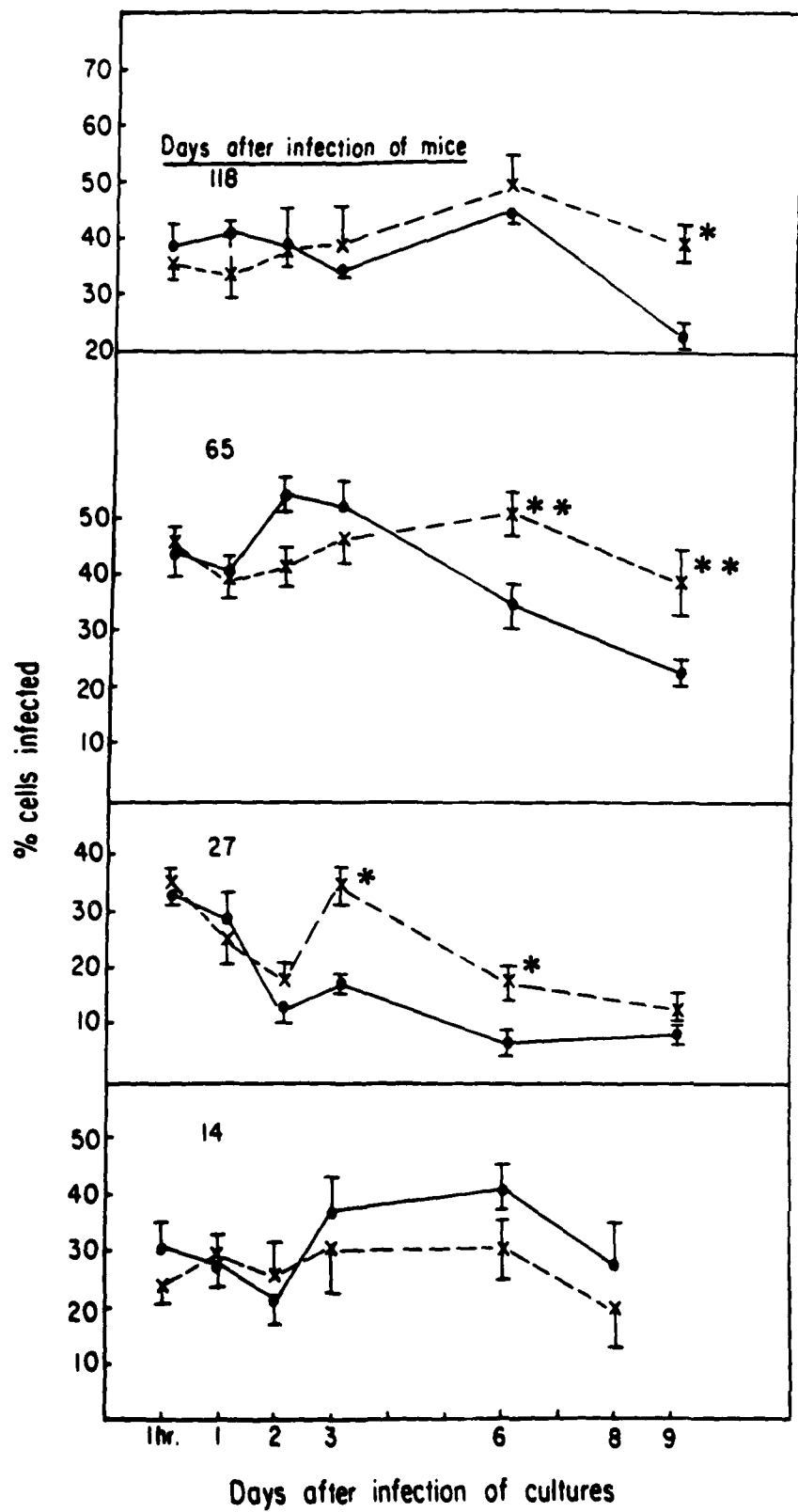
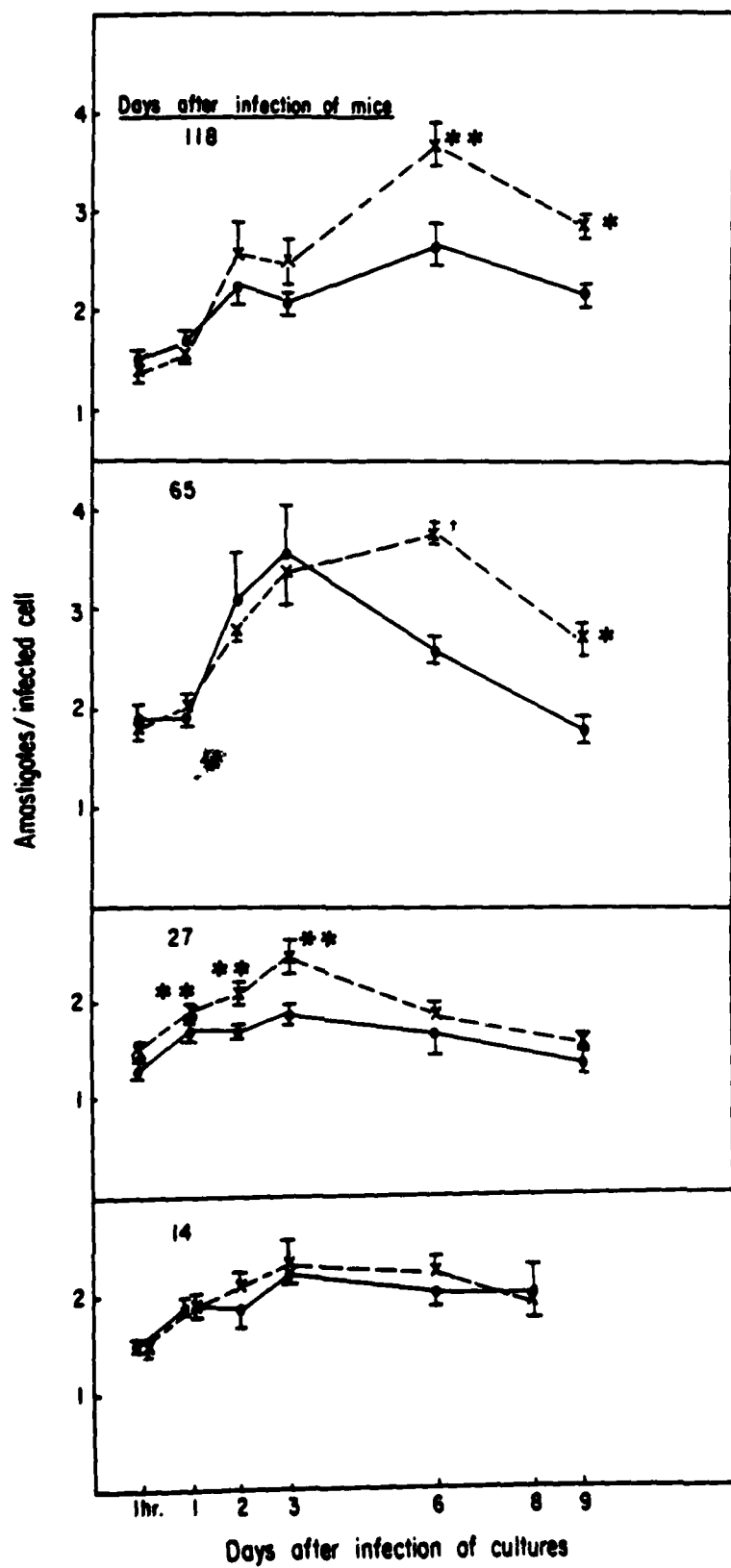
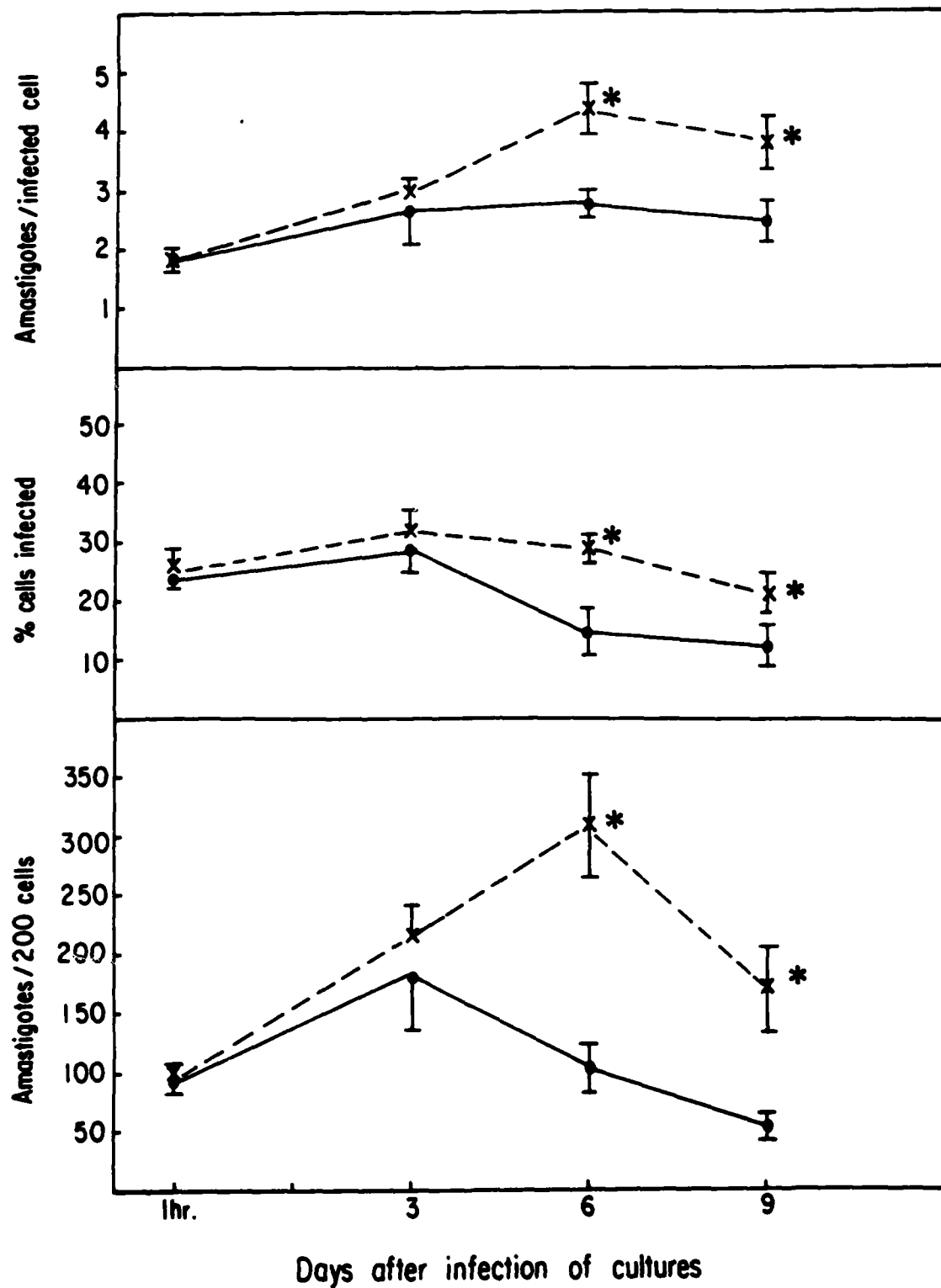


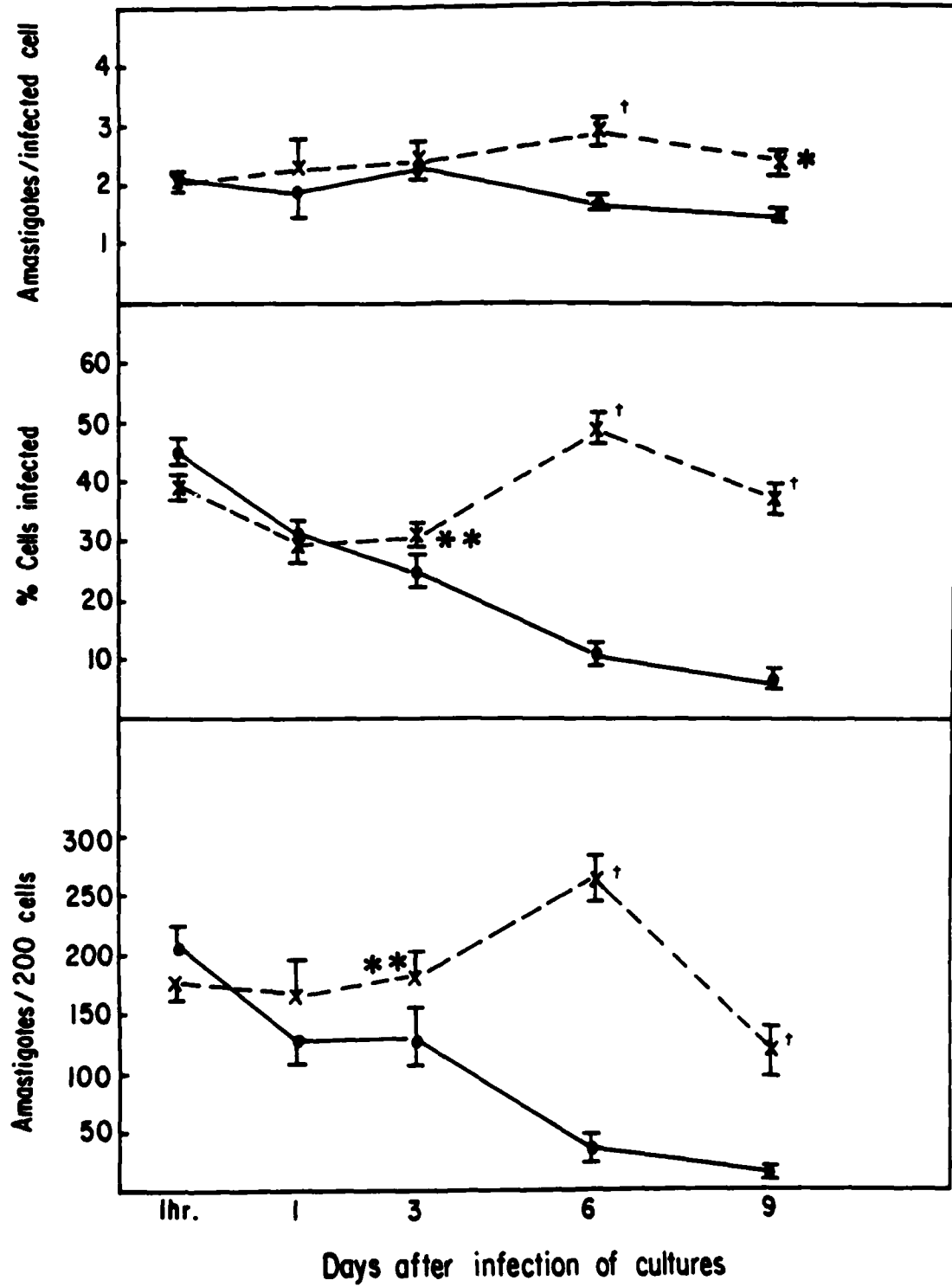
Fig 1

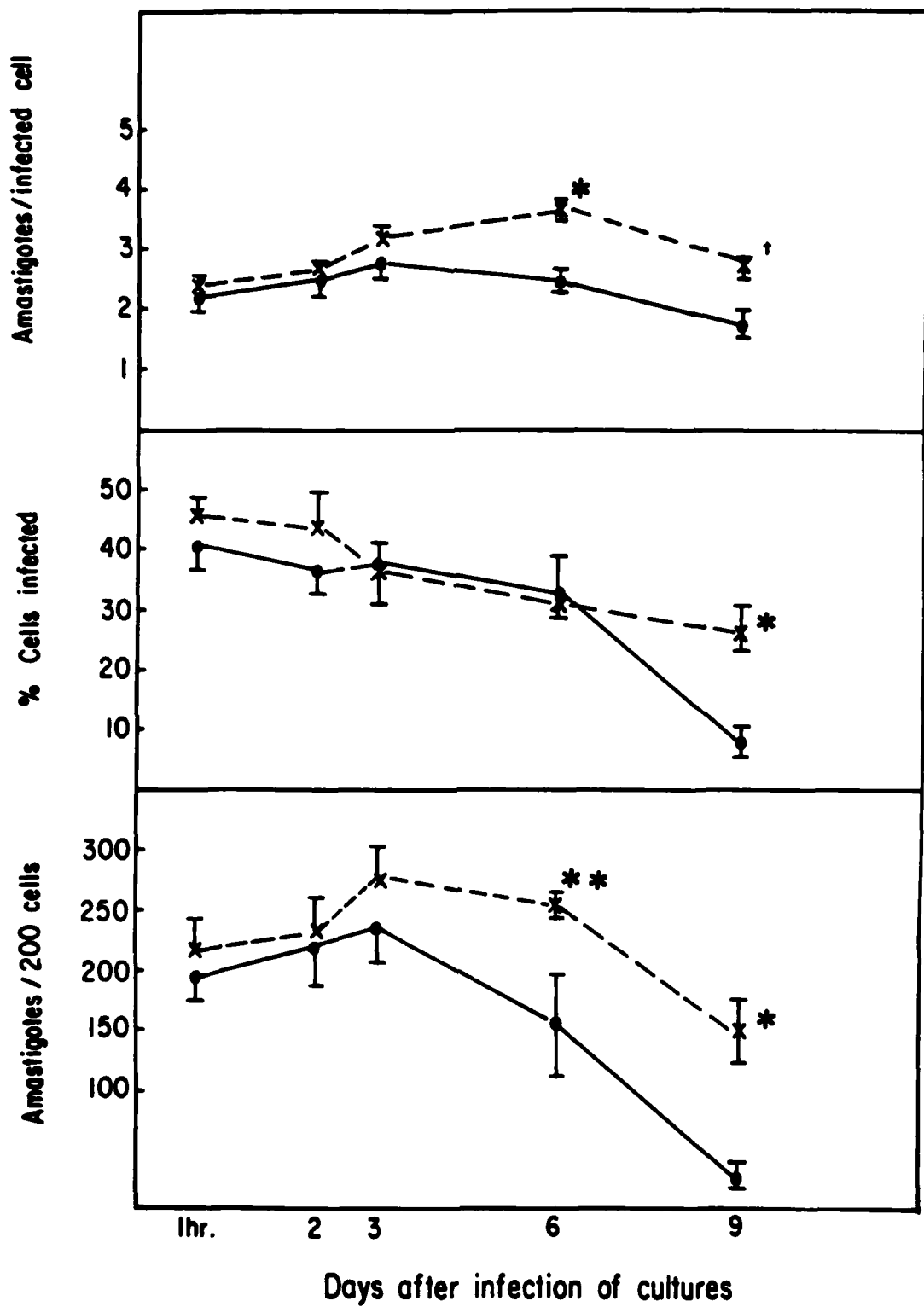












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